QIAAMP EXTRACTION OF BLOOD AND EPITHELIAL CELLS

A. SCOPE

This protocol employs the QIAamp DNA Mini Kit designed to extract DNA from epithelial cells and bloodstains that constitute a wide variety of forensic casework samples.

B. QUALITY CONTROL

B.1 Protective gloves, a lab coat, and a mask must be worn at all times when performing this procedure.

B.2 Each new QIAamp DNA Mini Kit lot must undergo quality control testing prior to extracting casework samples:

Biological material with known results along with a reagent control will be extracted using all the components of the kit undergoing quality control testing. The extracted material will be carried though the entire DNA analysis process. The results obtained from the known extracted sample must be as expected and good quality, as described in the GlobalFiler (DOC ID 12628) interpretation guidelines, for the kit to pass quality control testing. The quality control data will be placed into the critical reagent binder.

B.3 An analyst that dilutes the concentrated Buffers AW1 and AW2 prior to their initial use will be watched by a second individual from the Biology Unit to confirm correct preparation; this second individual can be another analyst, an Investigative Assistant, etc. Both individuals will initial the bottle. In addition, the lot number and expiration date of the added ethanol will be recorded on the bottle.

B.4 Refer to DOC ID 1835 to determine reagent expiration dates.

B.5 At least two reagent controls must be extracted along with a set of questioned samples.

B.6 In cases involving fetal tissue, in the absence of distinctive features, a minimum of five sections should be individually analyzed to obtain fetal DNA.

B.7 When consuming a sample and the corresponding extract, you must keep the post extraction substrate (refer to the DNA Quality Manual DOC ID 1833 for details on evidence consumption and retention).

C. SAFETY

C.1 Protective gloves, a lab coat, and a mask must be worn at all times when performing this procedure. Additionally, eye protection (e.g. safety glasses or a face shield) must be worn if this procedure is performed outside of a hood.

C.2 The sample preparation waste contains guanidine hydrochloride from Buffers AL and AW1, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilled, clean with water or ethanol.

C.3 All appropriate SDS sheets must be read prior to performing this procedure.

C.4 Treat all biological specimens as potentially infectious.

C.5 Distinguish all waste as general, biohazard, or sharps and discard appropriately.

D. REAGENTS, STANDARDS, AND CONTROLS

D.1 QIAamp DNA Mini Kit

D.1.1 Buffer ATL
D.1.2 Buffer AL
D.1.3 Proteinase K
D.1.4 Buffer AW1

Before using for the first time, add 125 mL ethanol (Absolute) to 95 mL AW1 concentrate

D.1.5 Buffer AW2

Before using for the first time, add 160 mL ethanol (Absolute) to 66 mL AW2 concentrate

D.1.6 Buffer AE

D.2 Absolute Ethanol (200 proof)

D.3 Bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner (Decontamination)

D.4 70% Reagent Alcohol (Decontamination)

E. EQUIPMENT & SUPPLIES

E.1 Equipment

E.1.1 Scissors/Forceps
E.1.2 Microcentrifuge
E.1.3 Eppendorf ThermoMixer
E.1.4 Eppendorf Smartblock 1.5 mL
E.1.5 Eppendorf Smartblock 2.0 mL
E.1.6 Eppendorf ThermoTop
E.1.7 Pipettes
E.1.8 Vortexer
E.1.9 Hood (optional)
E.1.10 UV Cross Linker

E.2 Supplies

E.2.1 Kimwipes
E.2.2 Microcentrifuge tubes
E.2.3 Spin baskets
E.2.4 Sterile aerosol resistant pipette tips
E.2.5 Microcentrifuge tube racks
E.2.6 Permanent marker
E.2.7 Disposable gloves
E.2.8 Mask
E.2.9 Lab coat
E.2.10 Eye protection (e.g. safety glasses, face shield)
E.2.11 Extraction sheet

F. PROCEDURE

*NOTE: You may double the volumes of reagents in step F.1 for samples that require larger liquid volumes. However, in order to maintain the correct reagent concentrations, you must double **all** reagent volumes in steps F.1-F.4.*

F.1 Cut the sample and place into a microcentrifuge tube. Add 200 µL ATL Buffer and 20 µL Proteinase K.

F.2 Incubate at approximately 56°C with approximately 550 rpm mixing for at least 1 hour and no longer than 24 hours using a ThermoMixer. This incubation time must be recorded and can be documented as a start and end time or total incubation time.

F.3 Briefly centrifuge to remove drops from inside the lid. Add 200 µL AL Buffer. Incubate at approximately 56°C with approximately 550 rpm mixing for 10 minutes using a ThermoMixer.

F.4 Briefly centrifuge to remove drops from inside the lid. Add 210 µL of Ethanol (200 proof). Vortex vigorously.

F.5 Briefly centrifuge the sample tube to remove drops from inside the lid.

For samples that typically contain a low amount of DNA, e.g. touched DNA samples, use a pipette tip or sterile forceps to remove the substrate and place it in a spin basket. Place basket back into tube and centrifuge to collect the fluid remaining in the substrate. Centrifuge the sample for 5 minutes at maximum speed. Add recovered liquid to a QIAamp spin column (the spin column holds up to 700 µL). Close the cap and centrifuge at approximately **6,800 rcf (8,000 rpm)** for 1 minute.

For samples that typically contain a large amount of DNA, e.g. reference samples, a spin basket may be used as described above, or carefully remove liquid from the microcentrifuge tube and add to the labeled QIAamp spin column without wetting the rim. Close the cap and centrifuge at approximately **6,800 rcf (8,000 rpm)** for 1 minute.

F.6 Place the QIAamp spin column in a clean collection tube and discard the tube containing the filtrate. Carefully open the spin column and add 500 µL AW1 Buffer without wetting the rim. Close the cap and centrifuge at approximately **6,800 rcf (8,000 rpm)** for 1 minute.

F.7 Place the QIAamp spin column in a clean collection tube and discard the tube containing the filtrate. Carefully open the spin column and add 500 µL AW2 Buffer without wetting the rim. Close the cap and centrifuge at approximately **20,800 rcf (14,000 rpm)** for 3 minutes.

F.8 Continue directly with step F.10, or if there is AW2 Buffer carryover, perform step F.9.

F.9 (Optional): Place the QIAamp spin column in a new collection tube and discard the collection tube with the filtrate. Centrifuge at approximately **20,800 rcf (14,000 rpm)** for 1 minute.

F.10 Place the QIAamp spin column in a clean labeled microcentrifuge tube (this will be the final storage tube) and discard the tube containing the filtrate. Carefully open the spin column and add 50 to 200 µL of AE Buffer and incubate at room temperature for at least 1 minute. Use a lower recovery volume (but not less than 50 µL) for samples that likely have low quantities of DNA.

F.11 Centrifuge at approximately **6,800 rcf (8,000 rpm)** for 1 minute.

F.12 Quantitate (DOC ID's 1784 and 1785) the DNA and concentrate samples (DOC ID 1780) as necessary. Alternatively, samples may be concentrated prior to quantitation. Store sample extracts in the refrigerator when not in use. Sample extracts may be frozen for long-term storage.

G. INTERPRETATION GUIDELINES

Not applicable

H. REFERENCES

H.1 QIAamp DNA Mini and Blood Mini Handbook. Third Edition, 04/2010.